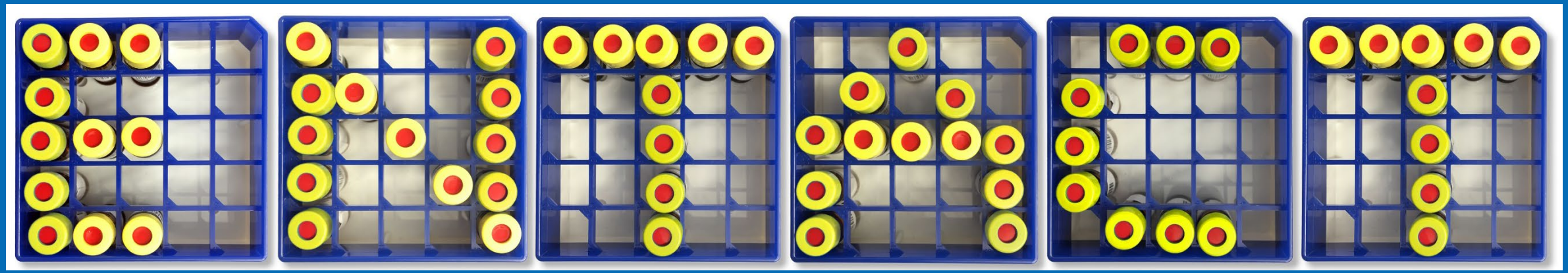
EPA
United States
Environmental Protection
Agency

Non-Targeted Analysis at the US EPA



The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.
Dust samples were determined to be environmental in nature and not human subjects research.

Exposure Science in the 21st Century: What are Key Drivers?

1) Understanding causes of disease

"…70-90% of disease risks are probably due to differences in environments"

EPIDEMIOLOGY

Environment and Disease Risks

A new paradigm is needed to assess how a lifetime of exposure to environmental factors affects the risk of developing chronic diseases.

Stephen M. Rappaport and Martyn T. Smith

Although the risks of developing chronic diseases are attributed to both genetic and environmental factors, 70 to 90% of disease risks are probably due to differences in environments (*1*–*3*). Yet, epidemiologists increasingly use genome-wide association studies (GWAS) to investigate diseases, while relying on questionnaires to characterize "environmental exposures." This is because GWAS represent the only approach for exploring the totality of any risk factor (genes, in this case) associated with disease prevalence. Moreover, the value of costly genetic information is diminished when inaccurate and imprecise environmental data lead to biased inferences regarding gene-environment interactions (*4*). A more comprehensive and quantitative view of environmental exposure is needed if epidemiologists are to discover the major causes of chronic diseases.

An obstacle to identifying the most important environmental exposures is the fragmentation of epidemiological research along lines defined by different factors. When epidemiologists investigate environmental risks, they tend to concentrate on a particular category of exposures involving air and water pollution, occupation, diet and obesity, stress and behavior, or types of infection. This slicing of the disease pie along parochial lines leads to scientific separation and confuses the definition of "environmental exposures." In fact, all of these exposure categories can contribute to chronic diseases and should be investigated collectively rather than separately.

To develop a more cohesive view of environmental exposure, it is important to recognize that toxic effects are mediated through chemicals that alter critical molecules, cells, and physiological processes inside the body. Thus, it would be reasonable to consider the "environment" as the body's internal chemical environment and "exposures" as the amounts of biologically active chemicals in this internal environment. Under this view, exposures are not restricted to chemicals (toxicants) entering the body from air, water, or food, for example, but also include chemicals produced by inflammation, oxidative stress, lipid peroxidation, infections, gut flora, and other natural processes (*5*, *6*) (see the figure). This internal chemical environment continually fluctuates during life due to changes in external and internal sources, aging, infections, life-style, stress, psychosocial factors, and preexisting diseases.

The term "exposome" refers to the totality of environmental exposures from conception onwards, and has been proposed to be a

School of Public Health, University of California, Berkeley, CA 94720–7356, USA. E-mail: srappaport@berkeley.edu

Published by AAAS

2) Ensuring chemical safety and human/eco health

GIVE A DOG A PHONE
Technology for our furry friends

NewScientist

WEEKLY November 29–December 5, 2014

We've made 150,000 new chemicals

We touch them, we wear them, we eat them

But which ones should we worry about?

SPECIAL REPORT, page 34

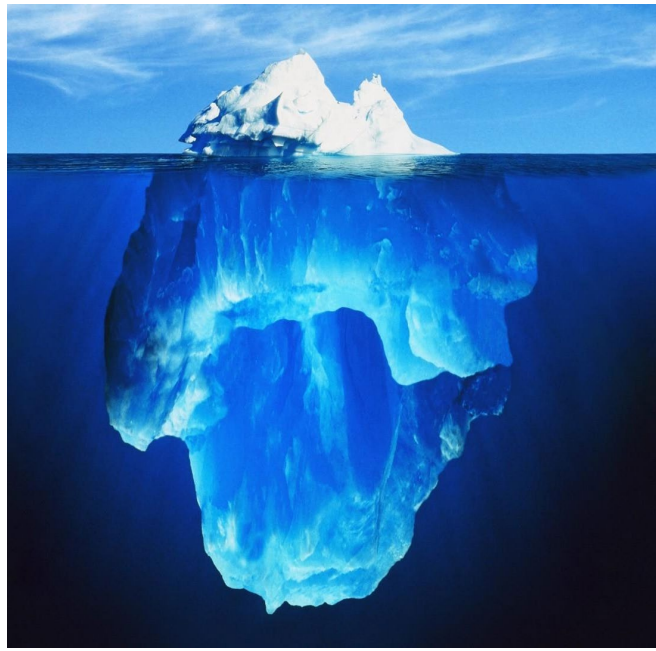
THE GOOD FIGHT
Most violence is also virtuous

CHAMBER OF SECRETS
The greatest ever find of early human bones

IS IT ALIVE?
Artificial worm could be first digital animal

Slide from Jon Sobus

What is Non-Targeted Analysis?

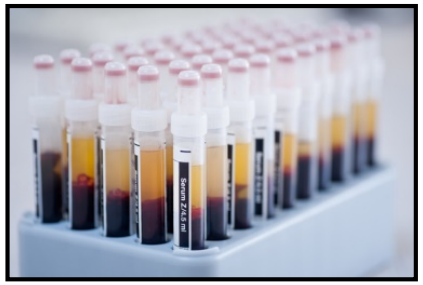


- **Targeted Analysis- the "known knowns"**
  - Covers <<1% of the exposome
  - Can't solve 21st century public health problems blinded to >99% of exposure data
- **Suspect Screening Analysis (SSA)- the "known unknowns"**
  - Covers ~5-10% of the exposome
  - Need rapid, efficient methods capable of measuring poorly studied compounds
- **Non-Targeted Analysis (NTA)-the "unknown unknowns"**
  - Covers 90-95% of the exposome
  - Need ways to characterize compounds that aren't yet known to exist

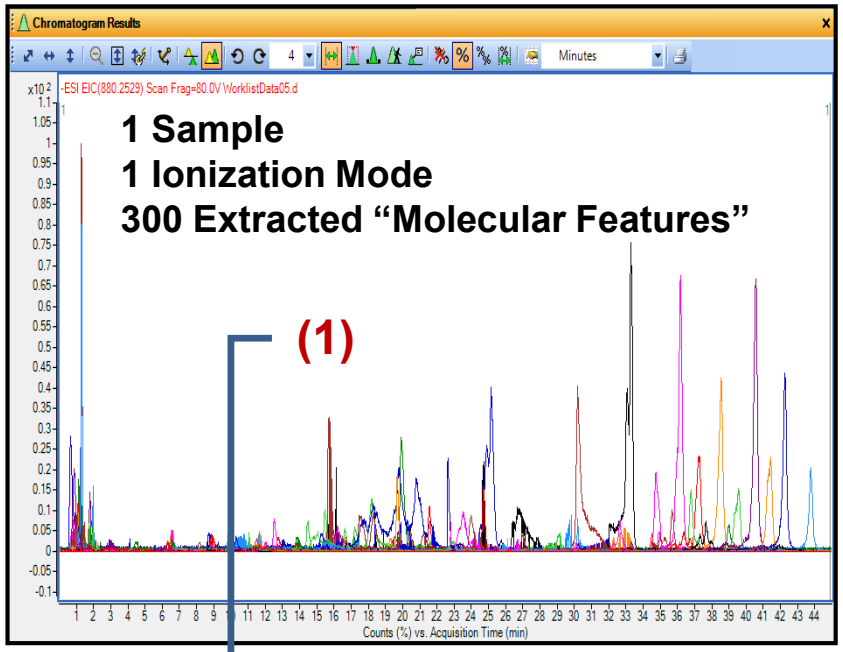
Slide from Elin Ulrich

NTA for Discovery of Unknowns

Samples



High-Resolution Mass Spec

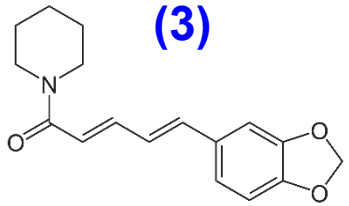


1) Prioritize "molecular features"
2) Correctly assign formulas
3) Correctly assign structures
4) Predict chemical concentrations
5) Determine chemical sources

(2)

$C_{17}H_{19}NO_3$

(3)



(4)

12 µg/g

(5)

Slide from Jon Sobus

NTA Applications at EPA

- **Exposure surveillance**
  - What chemicals are in food, water, products, dust, blood, etc.?
- **Chemical prioritization**
  - What are relevant chemicals & mixtures?
- **Exposure forensics**
  - What are chemical signatures of exposure sources?
- **Biomarker discovery**
  - What chemicals are associated with health impairment?

Slide from Jon Sobus

Chemical Surveillance in Consumer Products

Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2018, 52, 3125−3135

pubs.acs.org/est

Suspect Screening Analysis of Chemicals in Consumer Products

Katherine A. Phillips,[†] Alice Yau,[‡] Kristin A. Favela,[‡] Kristin K. Isaacs,[†] Andrew McEachran,[§,∥] Christopher Grulke,[∥] Ann M. Richard,[∥] Antony J. Williams,[∥] Jon R. Sobus,[†] Russell S. Thomas,[∥] and John F. Wambaugh*[,∥]

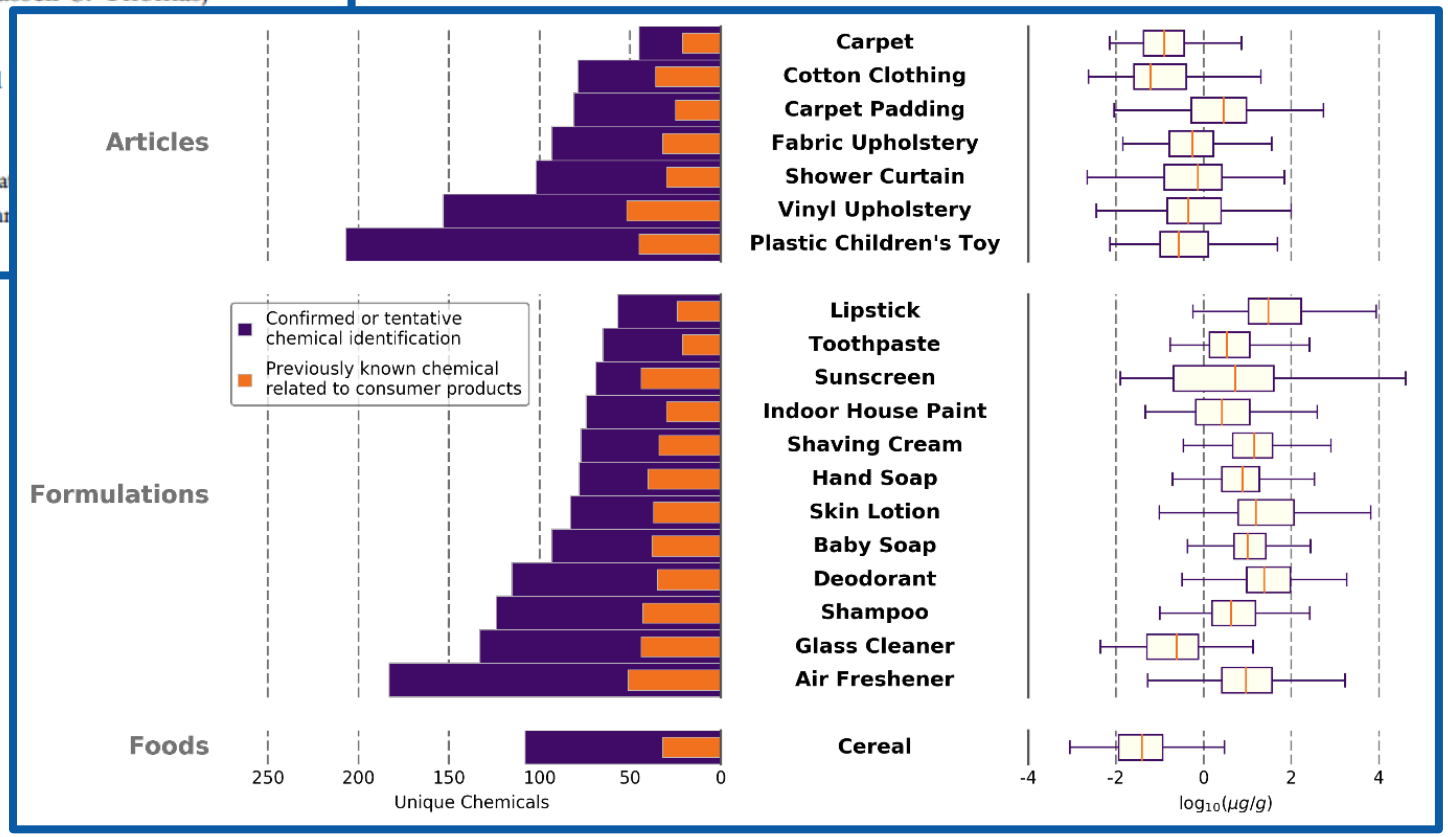
[†]National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Alexander Drive, Research Triangle Park, North Carolina 27711, United States

[‡]Southwest Research Institute, San Antonio, Texas 78238, United States

[§]Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, Tennessee 37830, United Sta

[∥]National Center for Computational Toxicology, Office of Research and Development, U.S. Environ T. W. Alexander Drive, Research Triangle Park, North Carolina 27711, United States

19% of chemicals identified by NTA are on consumer product chemical lists

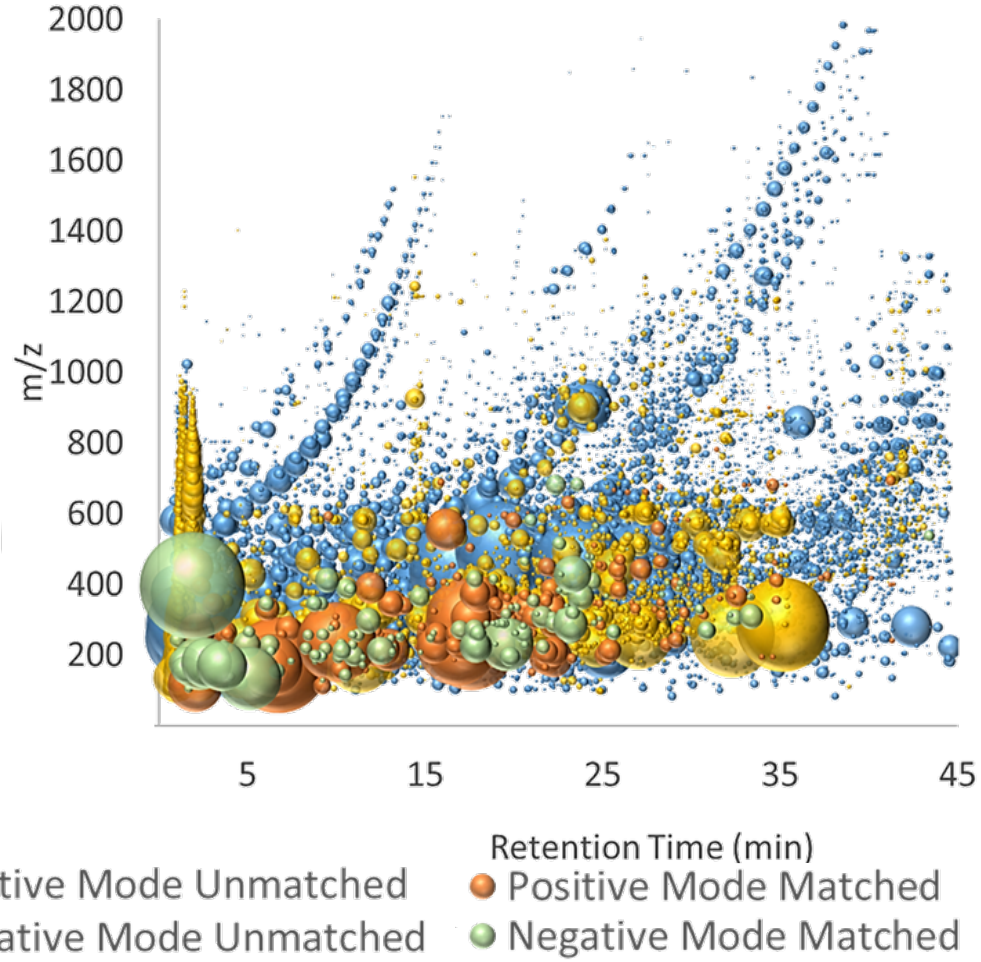


Slide from Jon Sobus

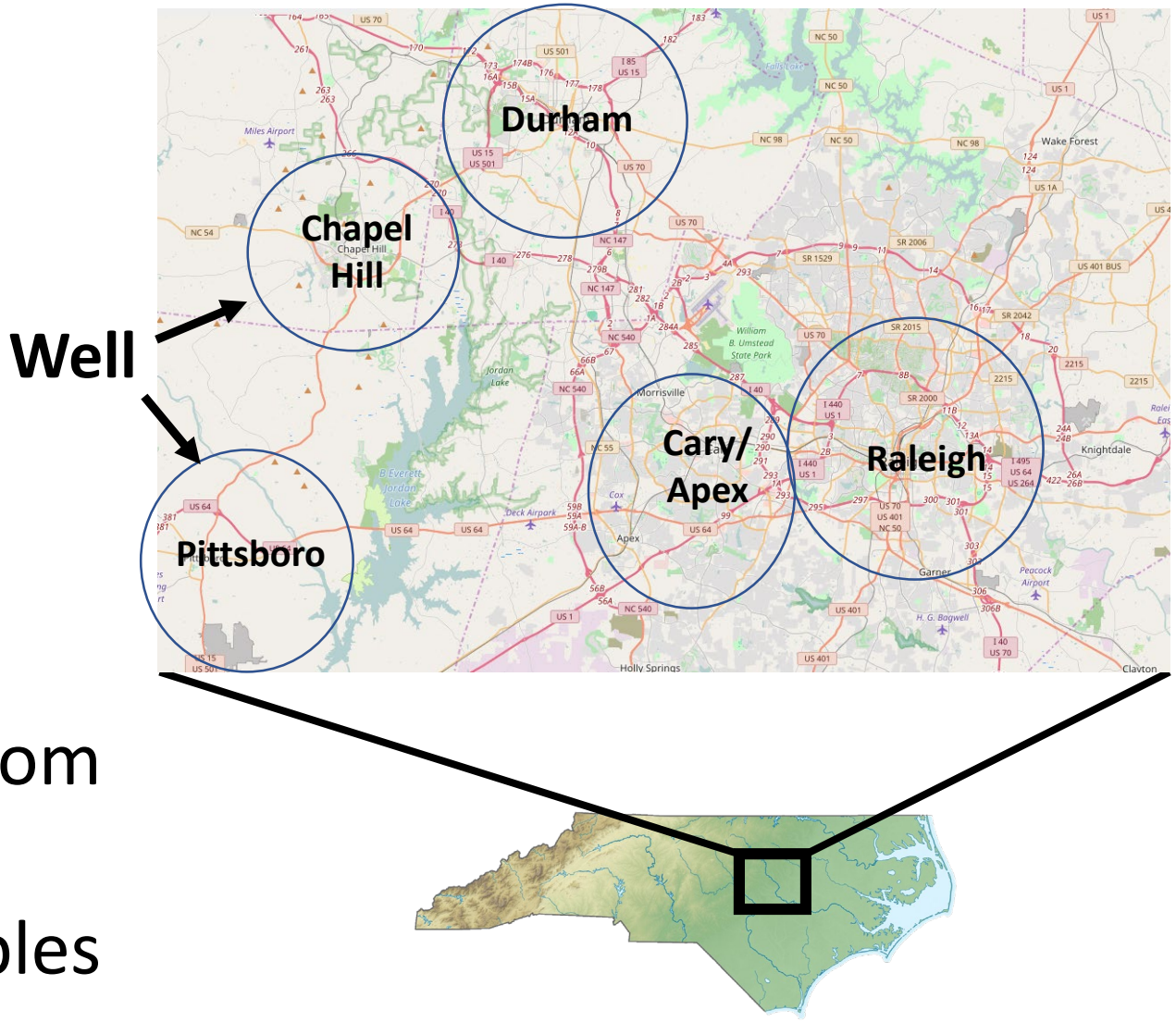
Screening of a large number of trace organic compounds in drinking water using point-of-use filters and suspect screening analysis

Seth R. Newton, Rebecca L. McMahen, Jon R. Sobus, Kamel Mansouri, Antony J. Williams, Andrew D. McEachran, and Mark J. Strynar

Office of Research and Development
National Exposure Research Lab
Research Triangle Park

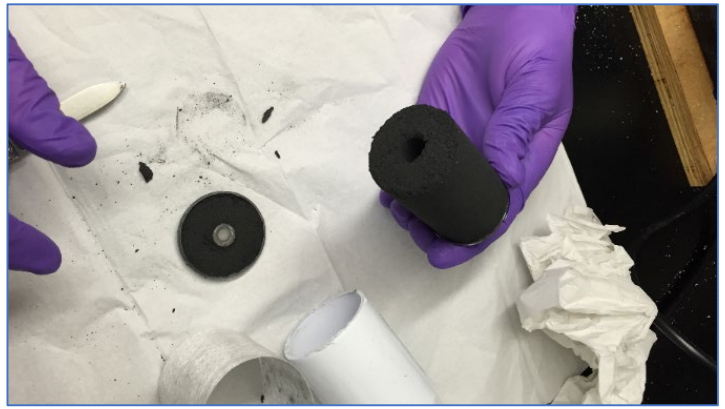


Sampling



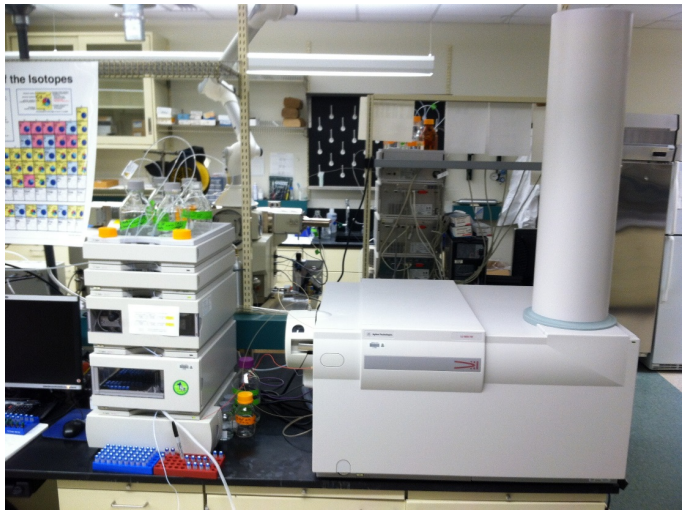
7 tap water samples from
5 municipalities
And 2 well water samples

Methods



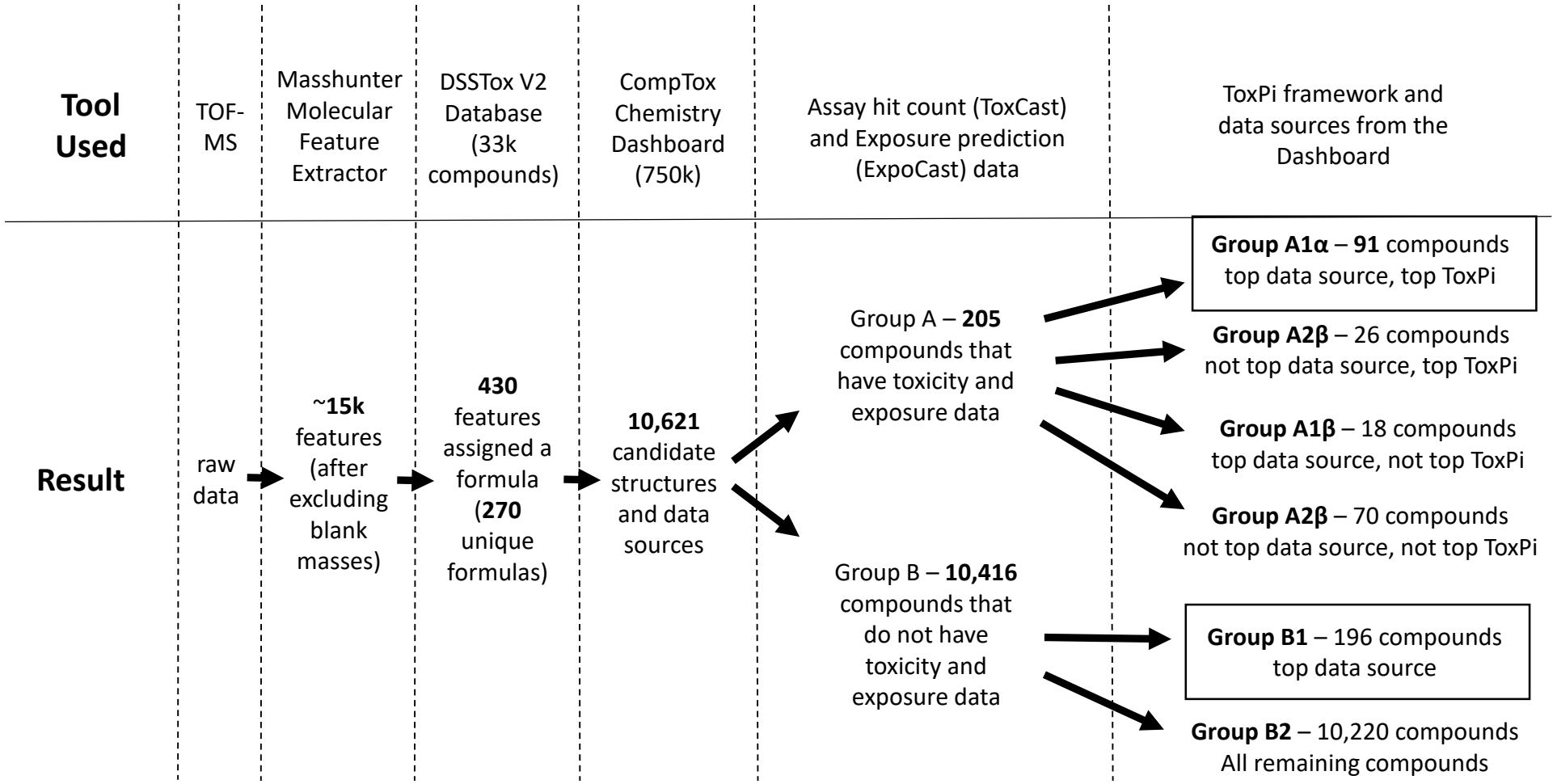
Soxhlet
DCM:MeOH
80:20

Volume reduction to 5 mL



Analysis by
HPLC-
Agilent 6210
TOF-HRMS

SSA Approach:



ToxPi Approach

ToxPi Score=

Abundance

+ Detection Frequency

+ Assay Hit Count

+ Exposure Prediction

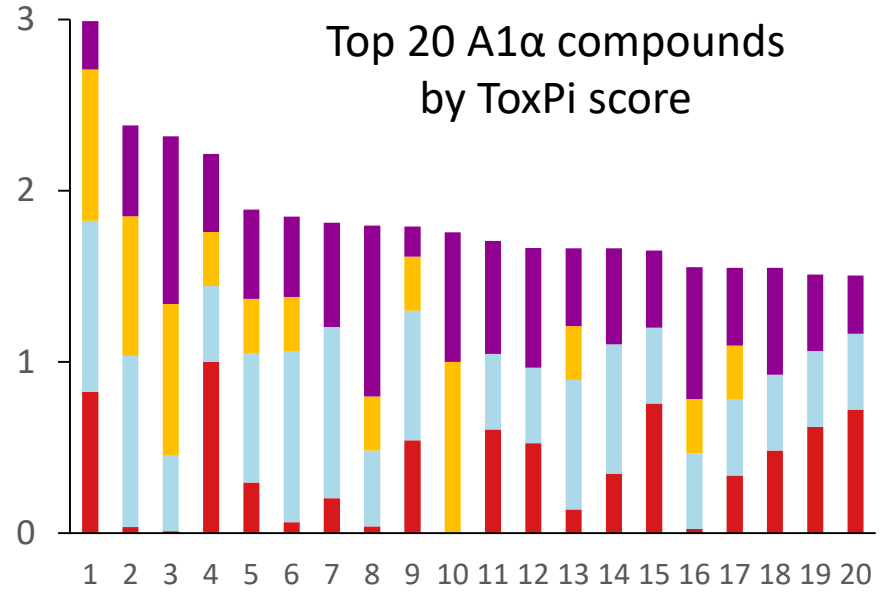
All terms normalized from 0 to 1

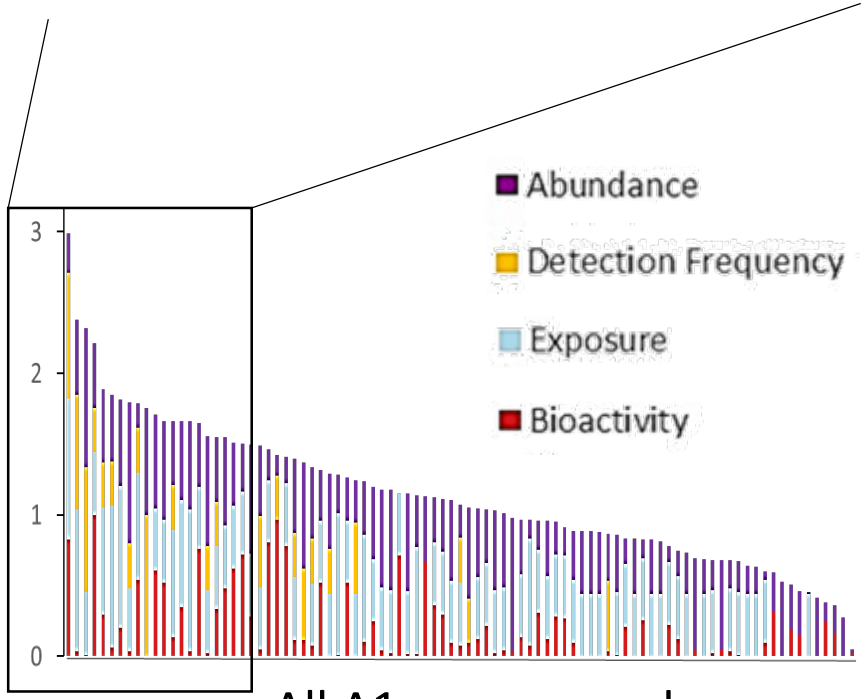
0 = minimum

and

1 = maximum

A1α ToxPi Scores





All A1α compounds

#	Compound	Toxpi Score
1	1,2-Benzisothiazolin-3-one*	2.99
2	Diethylene glycol	2.38
3	N-[3-(Dimethylamino)propyl] methacrylamide	2.32
4	Nonylparaben	2.22
5	Dipentyl phthalate	1.89
6	2-[2-(2-Butoxyethoxy) ethoxy]ethanol*	1.85
7	N,N-Dimethyldodecan-1-amine*	1.81
8	Sucralose	1.80
9	PFOS*	1.79
10	2-(2-Ethoxyethoxy) ethyl acetate*	1.76
11	TDCPP*	1.71
12	Zearalanol	1.67
13	PFOA*	1.66
14	Butylparaben	1.66
15	Noristerat	1.65
16	p-Synephrine	1.55
17	Alprostadil	1.55
18	Sclareol	1.55
19	PFDA*	1.51
20	Simvastatin	1.50

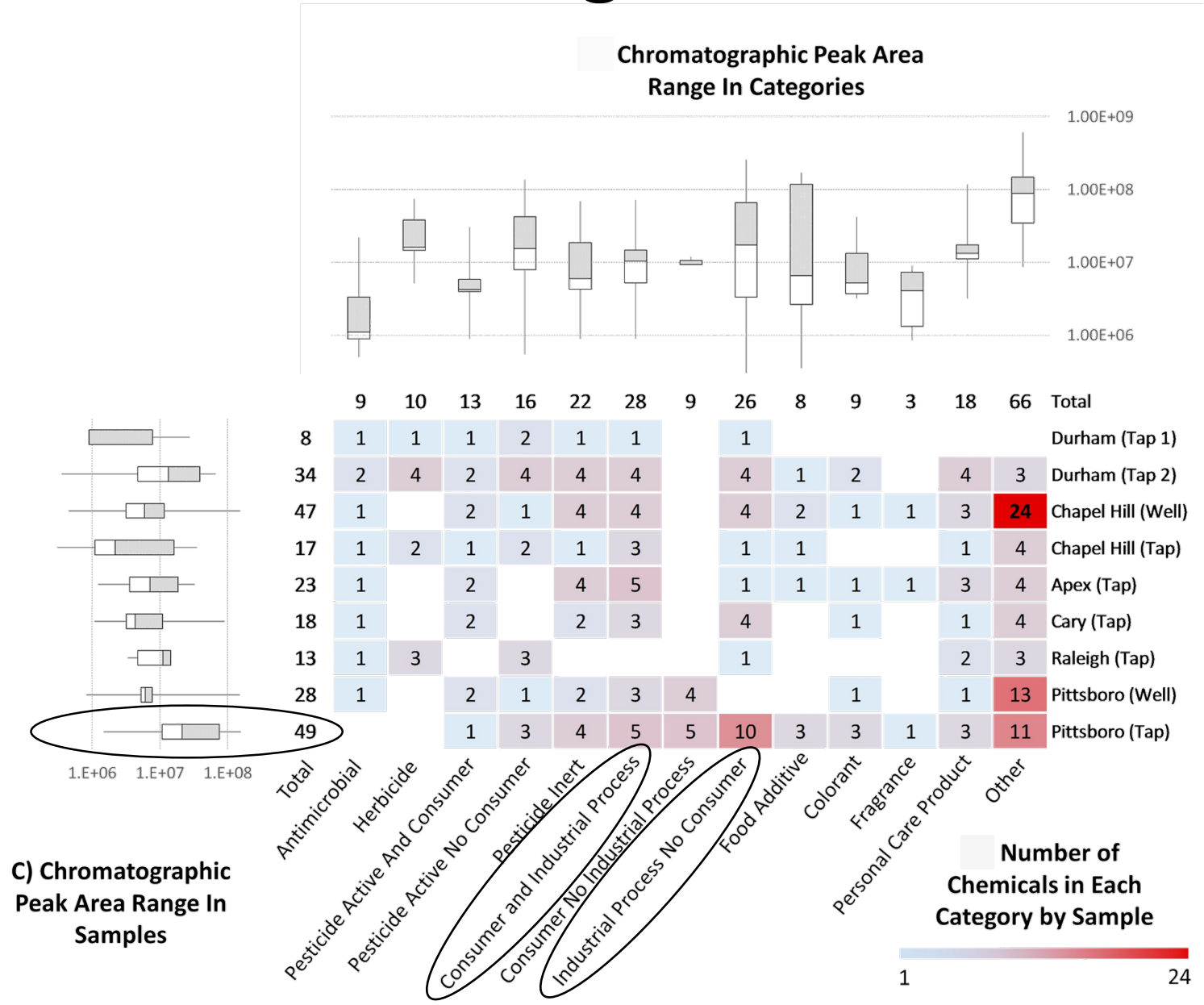
*Confirmed with standard

- 16 standards for 91 A1α compounds
- 15/16 true positives

1,2-Benzisothiazolin-3-one

- Many uses:
  - ➢ Preservative
  - ➢ Pesticide
- In 7/9 samples
- Active in 173 out of 565 Comptox assays

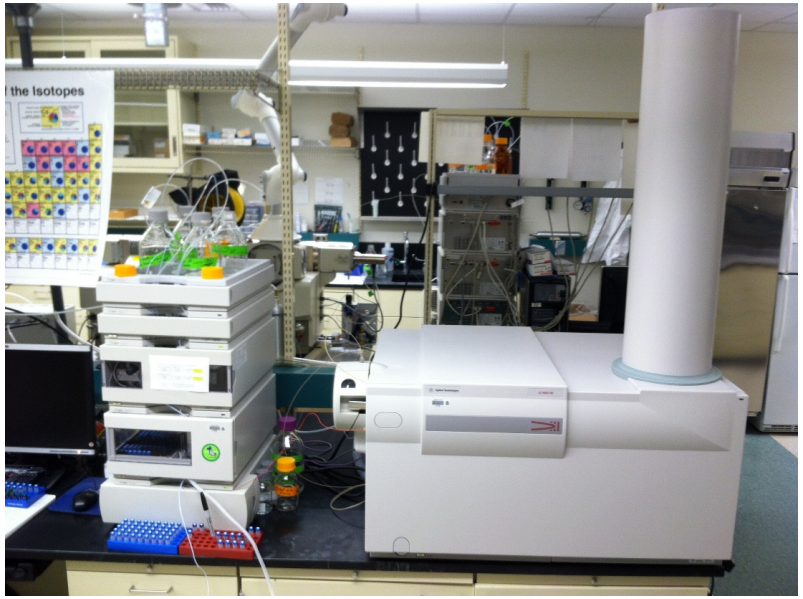
Product-Use Categories



C) Chromatographic Peak Area Range In Samples

Identifying Novel Polyfluorinated Alkyl Substances (PFASs) in the Tennessee River downstream of major manufacturing facilities in Decatur, Alabama

Seth Newton, James McCord, Rebecca McMahen, Andrew Lindstrom, James Stoeckel, Michael Chislock, Mark Strynar



Legacy PFASs in Decatur, Alabama

- Known contamination in the area from fluorochemical manufacturing facilities in Decatur

Environmental Science & Technology

ARTICLE

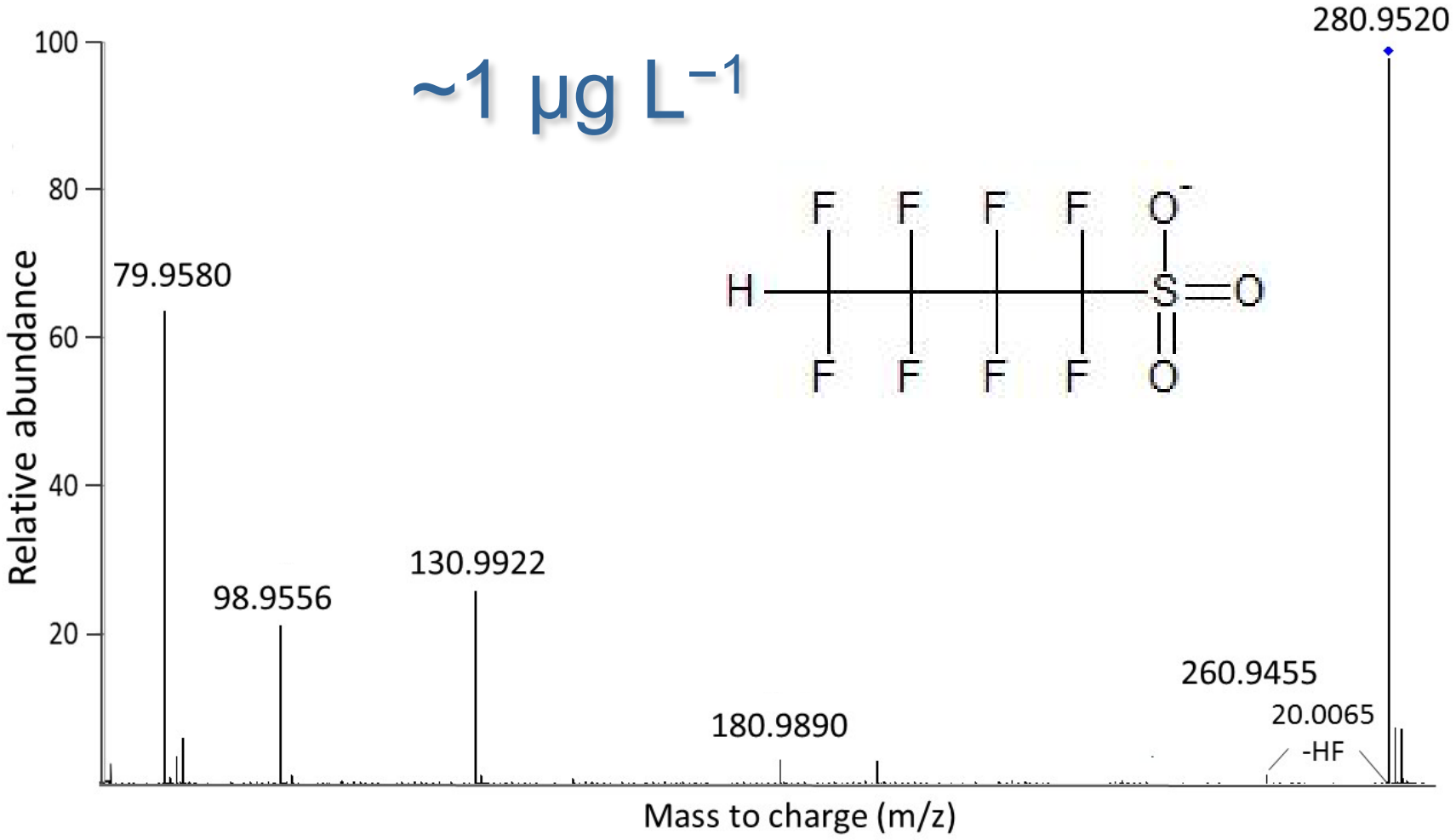
pubs.acs.org/est

Application of WWTP Biosolids and Resulting Perfluorinated Compound Contamination of Surface and Well Water in Decatur, Alabama, USA

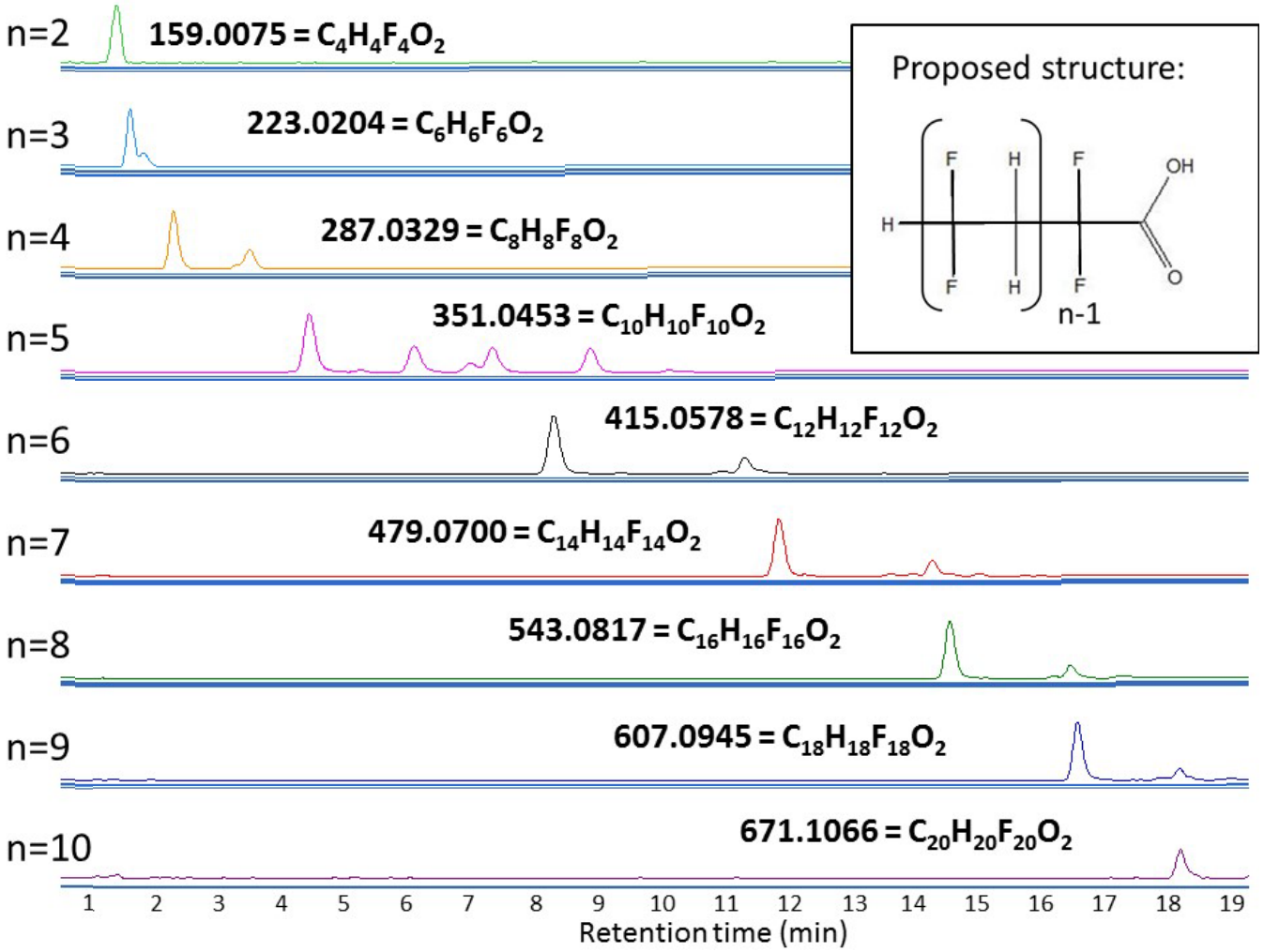
Andrew B. Lindstrom,[*,†] Mark J. Strynar,[†] Amy D. Delinsky,[†] Shoji F. Nakayama,[§] Larry McMillan,[‡] E. Laurence Libelo,[∥] Michael Neill,[⊥] and Lee Thomas[⊥]

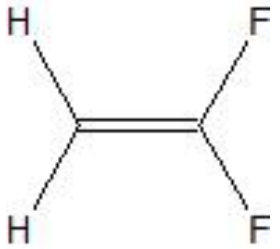
Single largest peak = 5x larger than PFBS peak

~1 µg L^{-1}



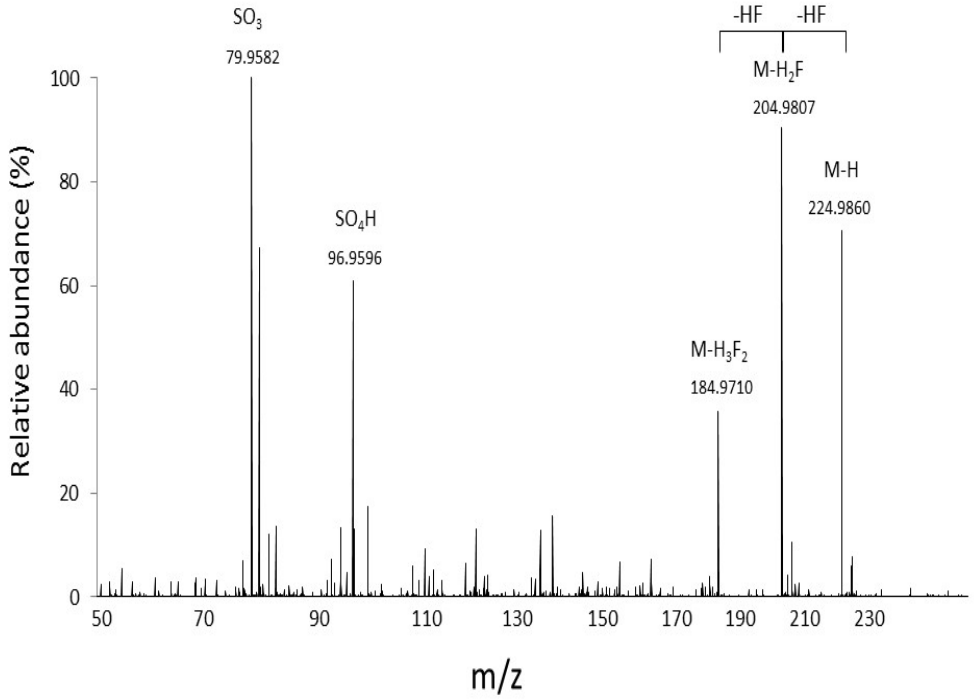
Carboxylic Acid Series



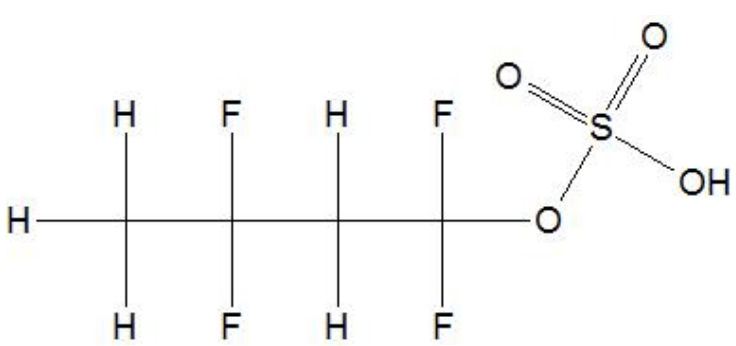


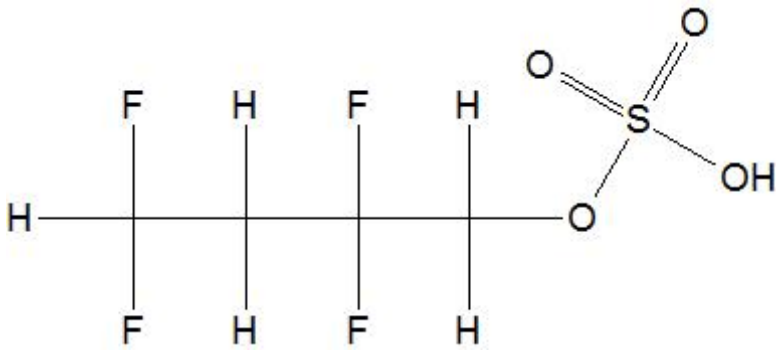
- Registered in TSCA for 3M in Decatur
- See a CO_2 loss in the spectrum
- Likely byproduct of Polyvinylidene fluoride (PVDF) production

An Unknown Polyfluorinated Sulfate

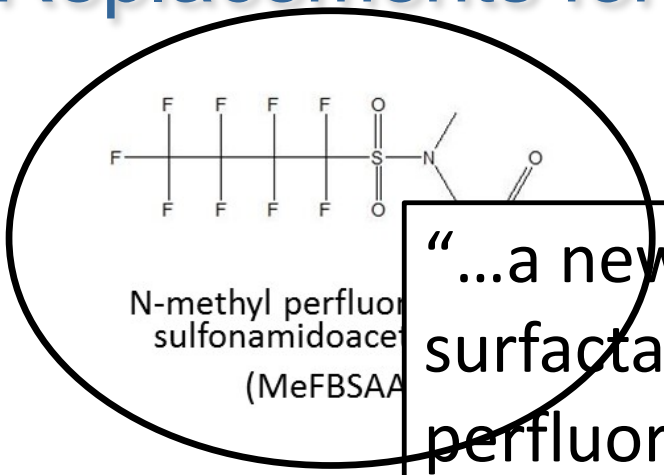


Proposed structures for 224.9860 m/z:

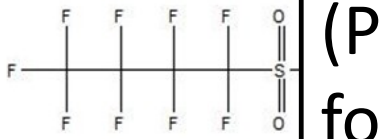




Perfluorobut
Replacements for Pe



N-methyl perfluor
sulfonamidoacet
(MeFBSAA



perfluorobut
sulfonamidoacetic
acid

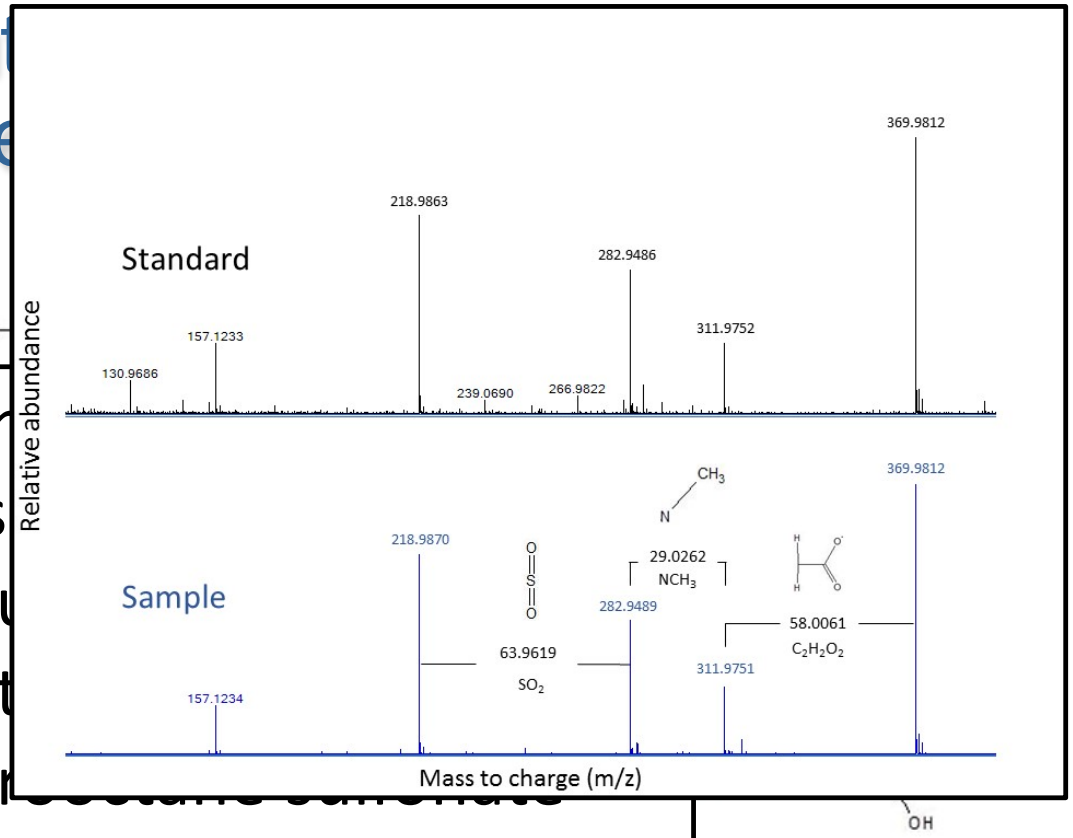
(FBSAA)

sulfonamidoacetic acid

(EtFBSAA)

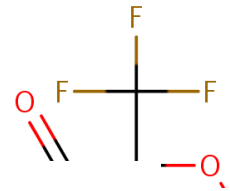
thyl
perfluorobutane
sulfonamdoethanol

(MeFBSE)



"…a new lin
surfactants
perfluorobu
(PFBS)…pot
for perfluor
(PFOS) based surfactants." – 3M

The GenX Saga



GenX Inv

The News & Observer

SECT

ehp Environmental Health Perspectives

OPEN ACCES

HOME | CURRENT ISSUE | ARCHIVES | COLLECTIONS | 中文翻译 | AUTHORS | ABOUT

Open Access

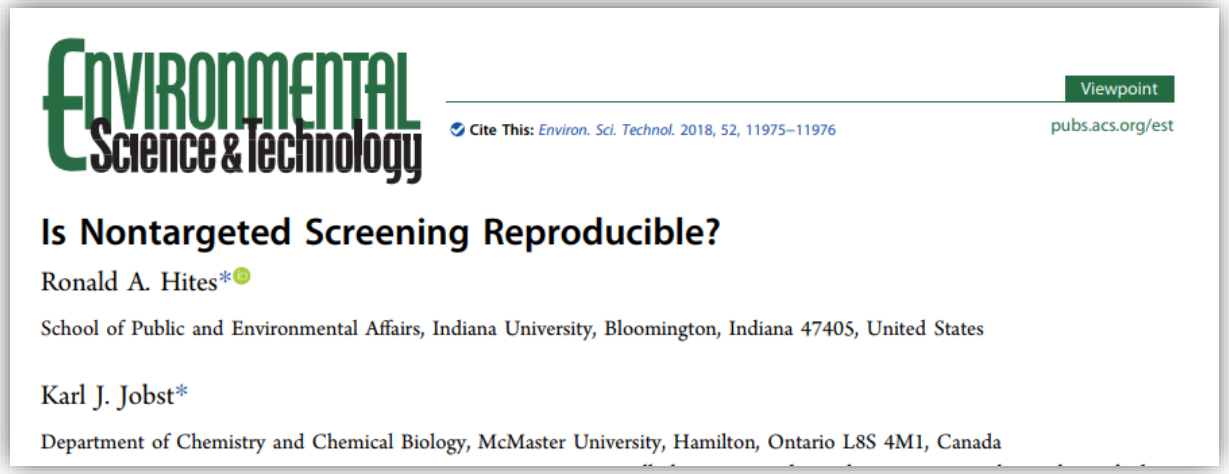
NEWS

Deal reached for Chemours to stop remaining GenX chemical pollution of Cape Fear River

Paul Woolverton The Fayetteville Observer

Published 7:51 p.m. ET Aug. 13, 2020 | **Updated 12:15 p.m. ET Aug. 14, 2020**

NTA State-of-the-Science



Environmental Science & Technology

Viewpoint

Cite This: *Environ. Sci. Technol.* 2018, 52, 11975−11976

pubs.acs.org/est

Is Nontargeted Screening Reproducible?

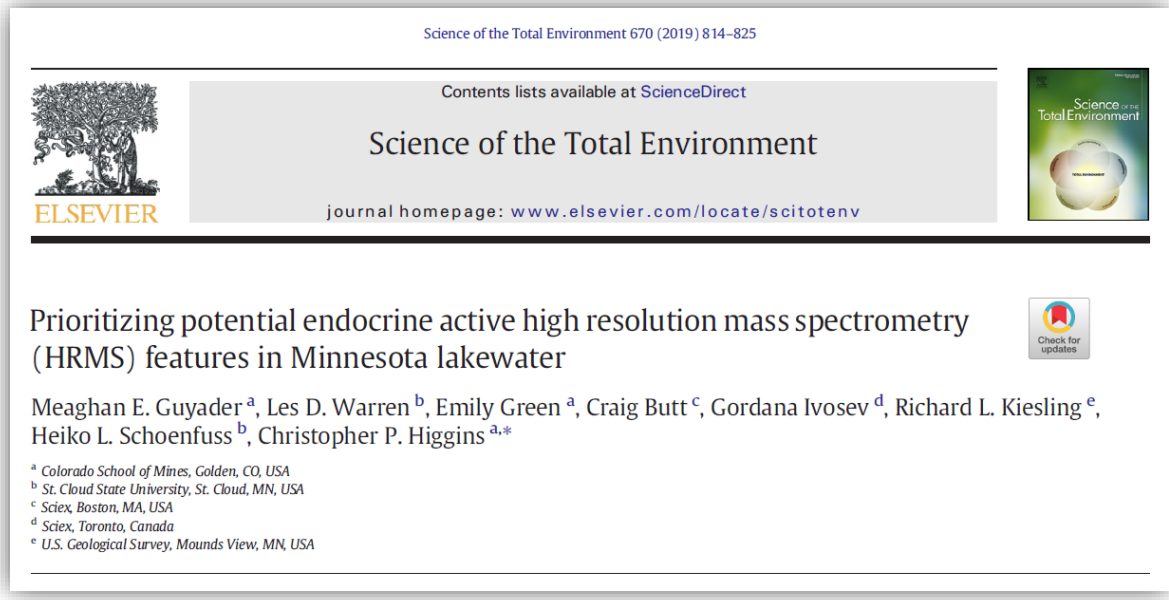
Ronald A. Hites*

School of Public and Environmental Affairs, Indiana University, Bloomington, Indiana 47405, United States

Karl J. Jobst*

Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario L8S 4M1, Canada

"No single analytical technique is suitable for the analysis of all compounds, and successful nontargeted screening will require the development of multiplatform approaches, facilitated and validated through interlaboratory collaborations."



Science of the Total Environment 670 (2019) 814–825

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

ELSEVIER

Prioritizing potential endocrine active high resolution mass spectrometry (HRMS) features in Minnesota lakewater

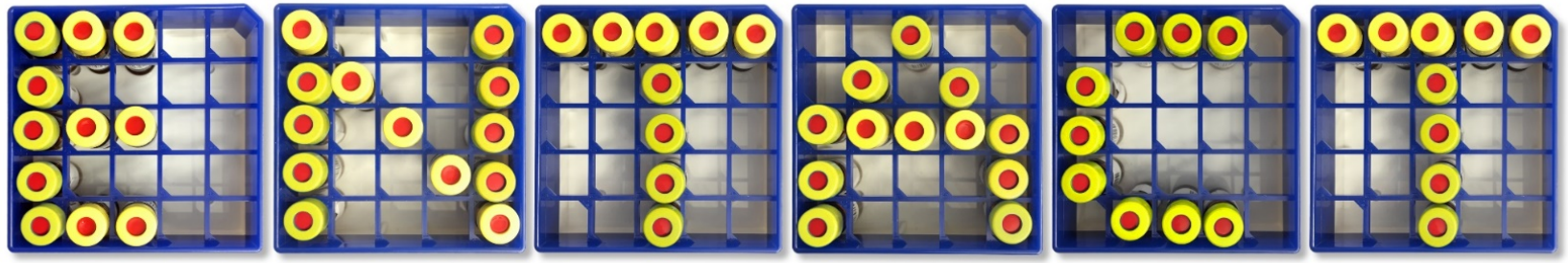
Meaghan E. Guyader [a], Les D. Warren [b], Emily Green [a], Craig Butt [c], Gordana Ivosev [d], Richard L. Kiesling [e], Heiko L. Schoenfuss [b], Christopher P. Higgins [a,*]

[a] Colorado School of Mines, Golden, CO, USA
[b] St. Cloud State University, St. Cloud, MN, USA
[c] Sciex, Boston, MA, USA
[d] Sciex, Toronto, Canada
[e] U.S. Geological Survey, Mounds View, MN, USA

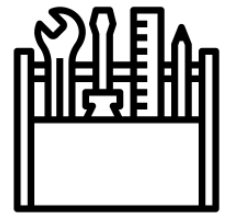
"The novelty of nontarget analysis, particularly its current lack of implementation by regulatory agencies, has prevented the establishment of streamlined quality assurance and quality control (QA/QC) procedures."

Slide from Jon Sobus

EPA's Non-Targeted Analysis Collaborative Trial



- How variable are tools and results from lab to lab?
- Are some methods/tools better than others?
- How does sample complexity affect performance?
- What chemical space does a given method cover?
- How sensitive are specific instruments/methods?

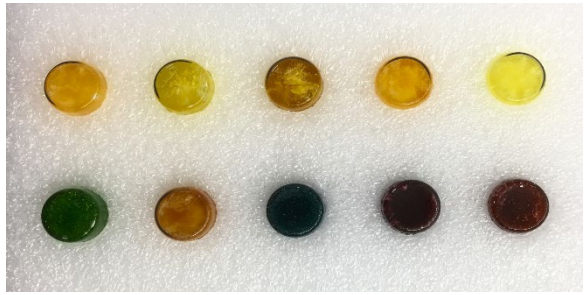


Slide from Jon Sobus

ENTACT Sample Overview

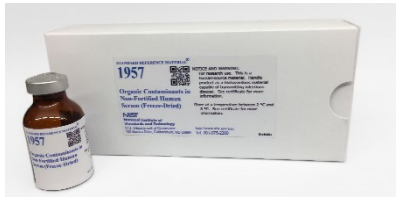
Part 1. Ten ToxCast mixtures

95, 185 or 365 substances/mixture

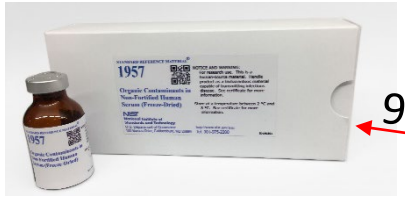


Part 2. Three standardized exposure relevant extracts

Unaltered

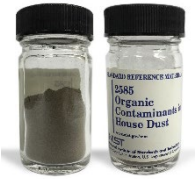


Fortified



NIST SRM 1957-
Organic Contaminants in Non-fortified Human Serum

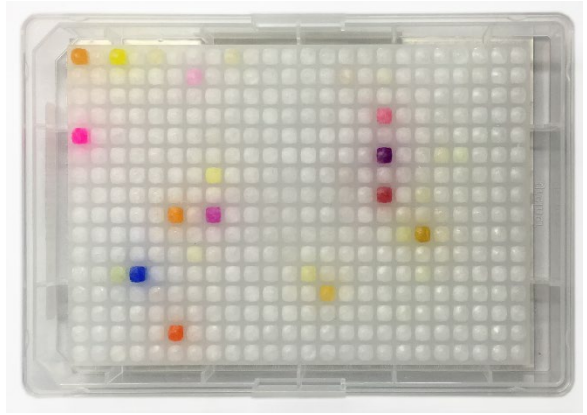
Oregon State University-
Outdoor air exposed silicone wrist-bands



NIST SRM 2585-
Organic Contaminants in House Dust

Part 3. Individual ToxCast standards

1,269 ENTACT; 4,685 ToxCast all



Slide from Jon Sobus

Who Else is Working on ENTACT?

Contractors:

University of Alberta
Emory University
Duke University
Oregon State University
San Diego State University
Pacific Northwest National Laboratory

19 Blind submissions

15 Unblinded submissions

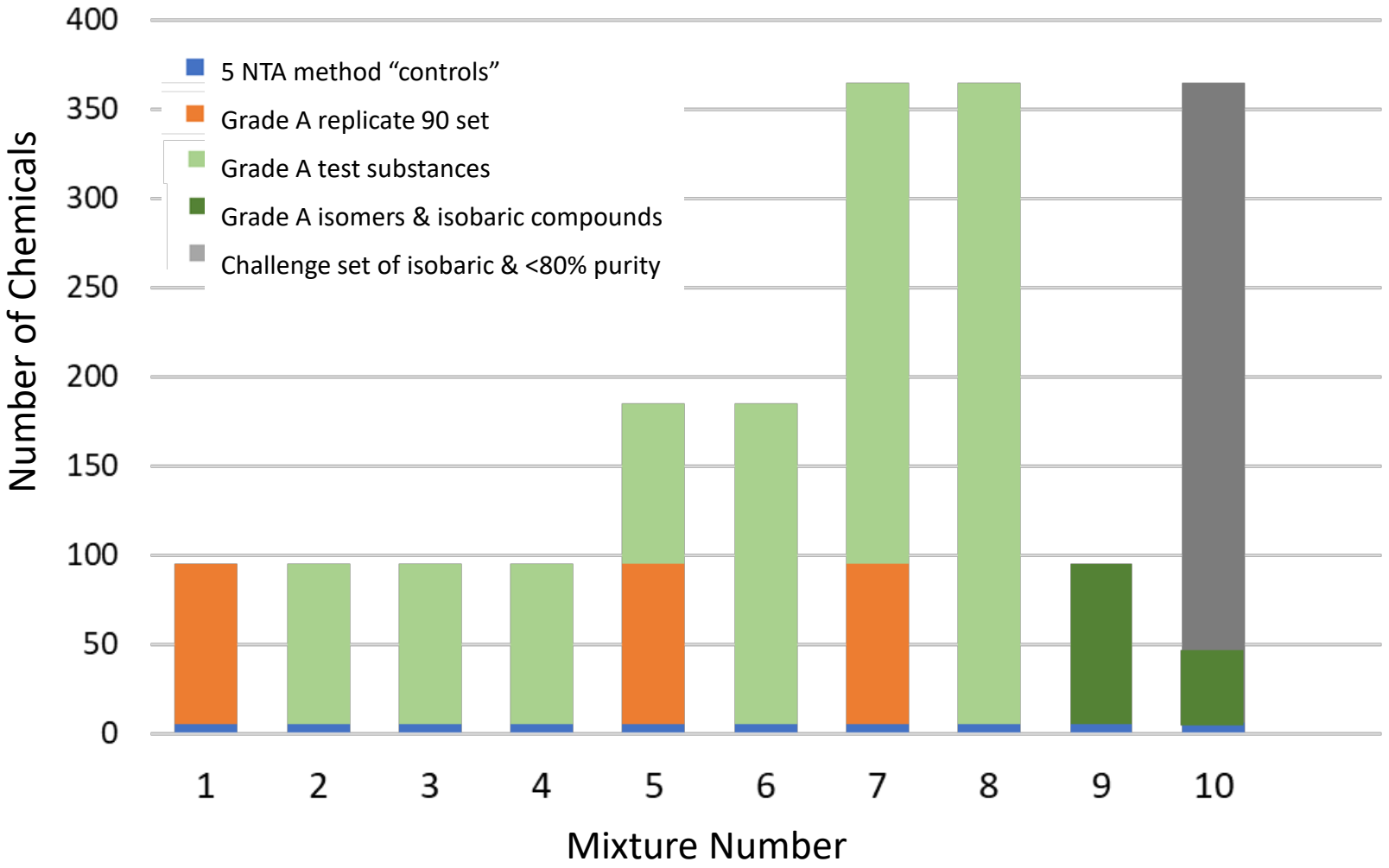
Vendors:

The Scripps Research Institute
Agilent Technologies
Thermo Scientific
Leco
AB SCIEX
Waters

General Participants:

uni.lu Université du Luxembourg
UF University of Florida
Wisconsin State Laboratory of Hygiene, University of Wisconsin-Madison
NIST
NC State University
Recetox
Cornell University
UW Tacoma
Mount Sinai
University of Birmingham
CalEPA California Environmental Protection Agency
eawag aquatic research
United States Environmental Protection Agency
FIU

ENTACT Mixtures- Brainchild of C. Grulke



<u>10 Prepared Mixtures</u>:

1,939 total spiked substances

1,269 unique substances:

1 → spiked 11 times

4 → spiked 10 times

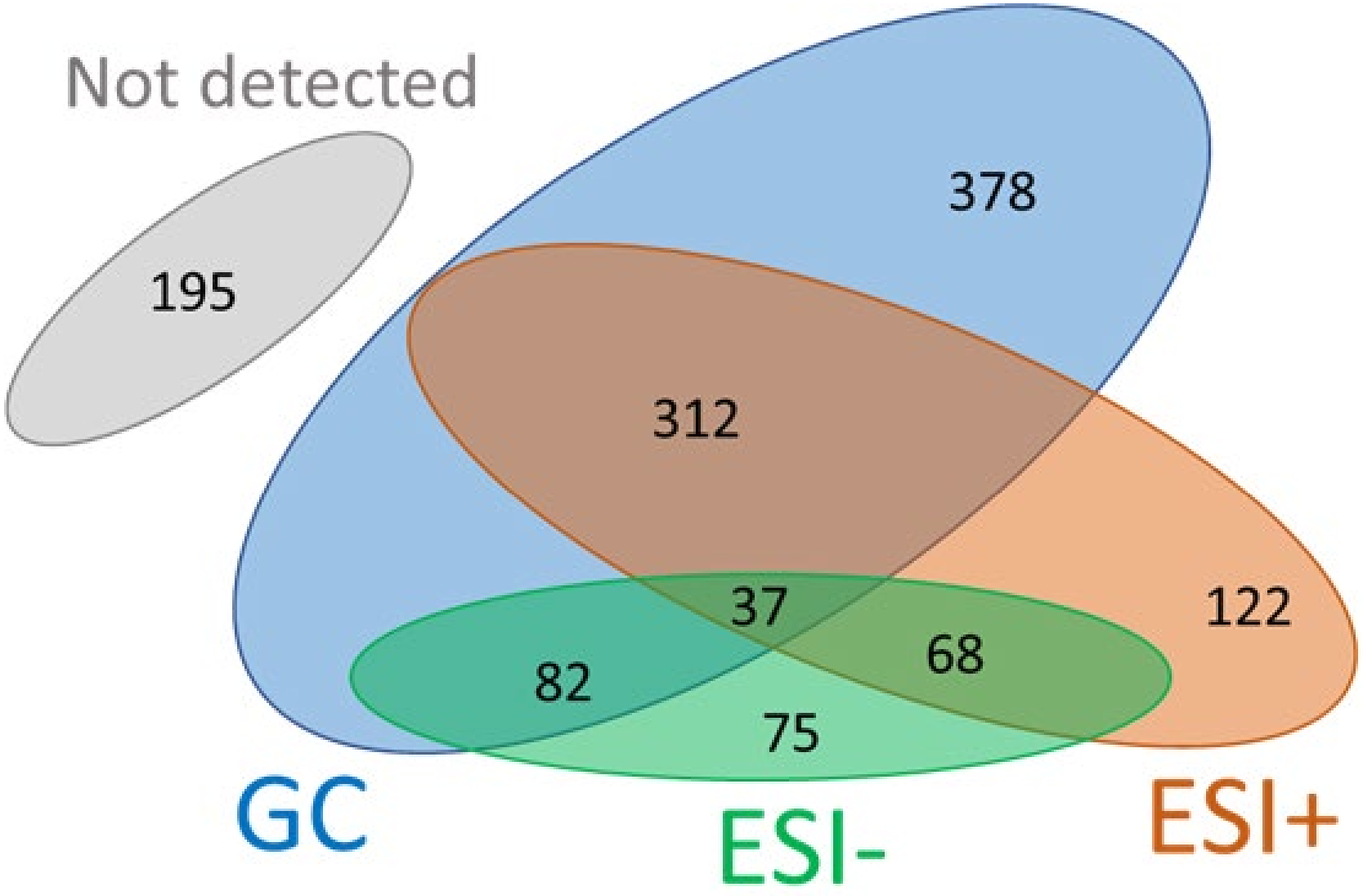
57 → spiked 4 times

33 → spiked 3 times

388 → spiked 2 times

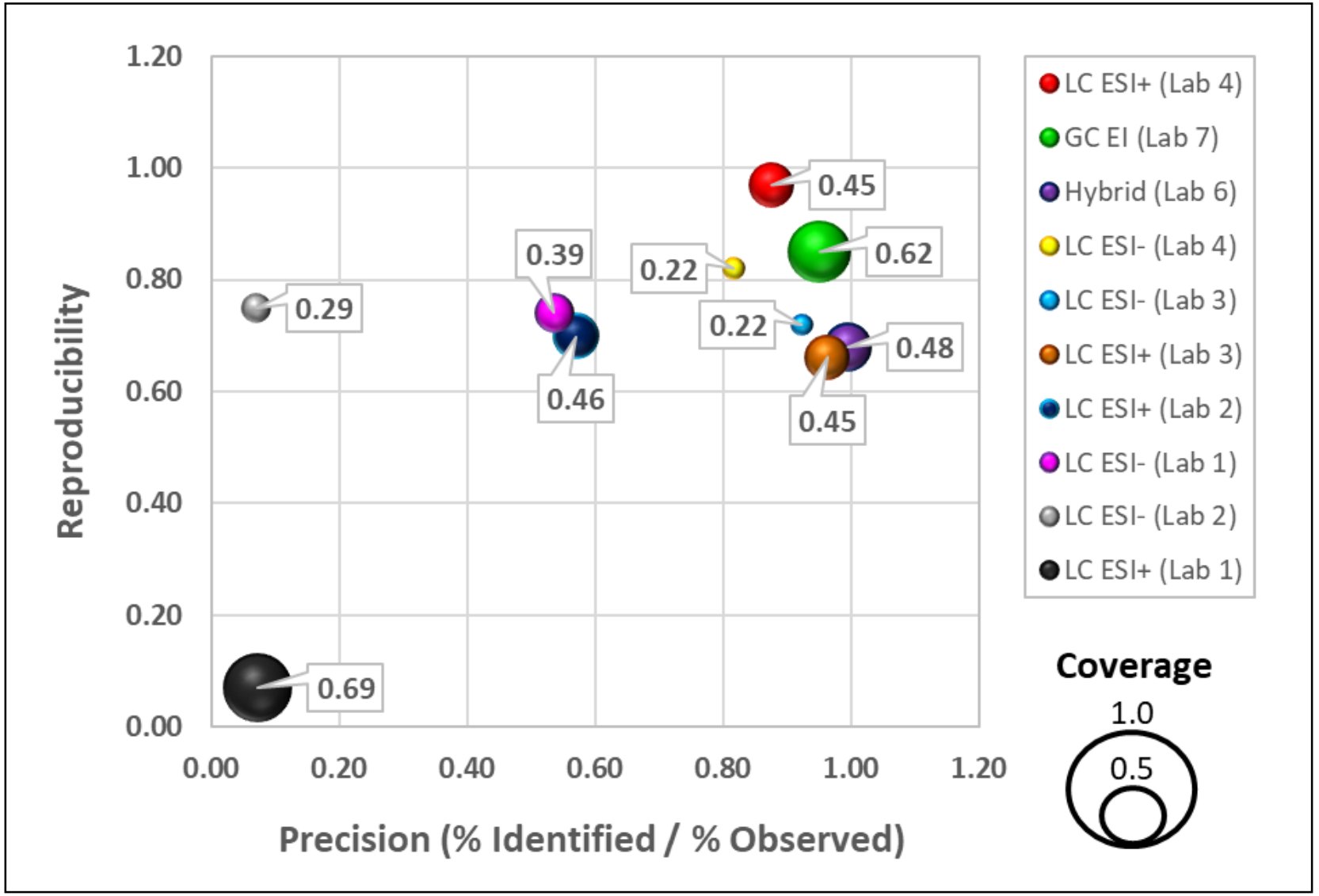
786 → spiked 1 time

ENTACT Initial Results: Method Coverage



Ulrich EM, et al. (2019) *ABC* 411:853-866. doi:10.1007/s00216-018-1435-6

Lab Comparison: Total Performance



Metrics (all %):

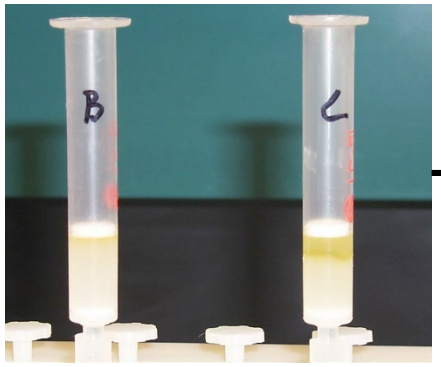
X-Axis →
How often correct?

Y-Axis →
How consistent?

Bubble Size →
How much coverage?

Dust Spiking Experiment

Sonication
Methanol



Silica > Methanol

C. Extract Spike

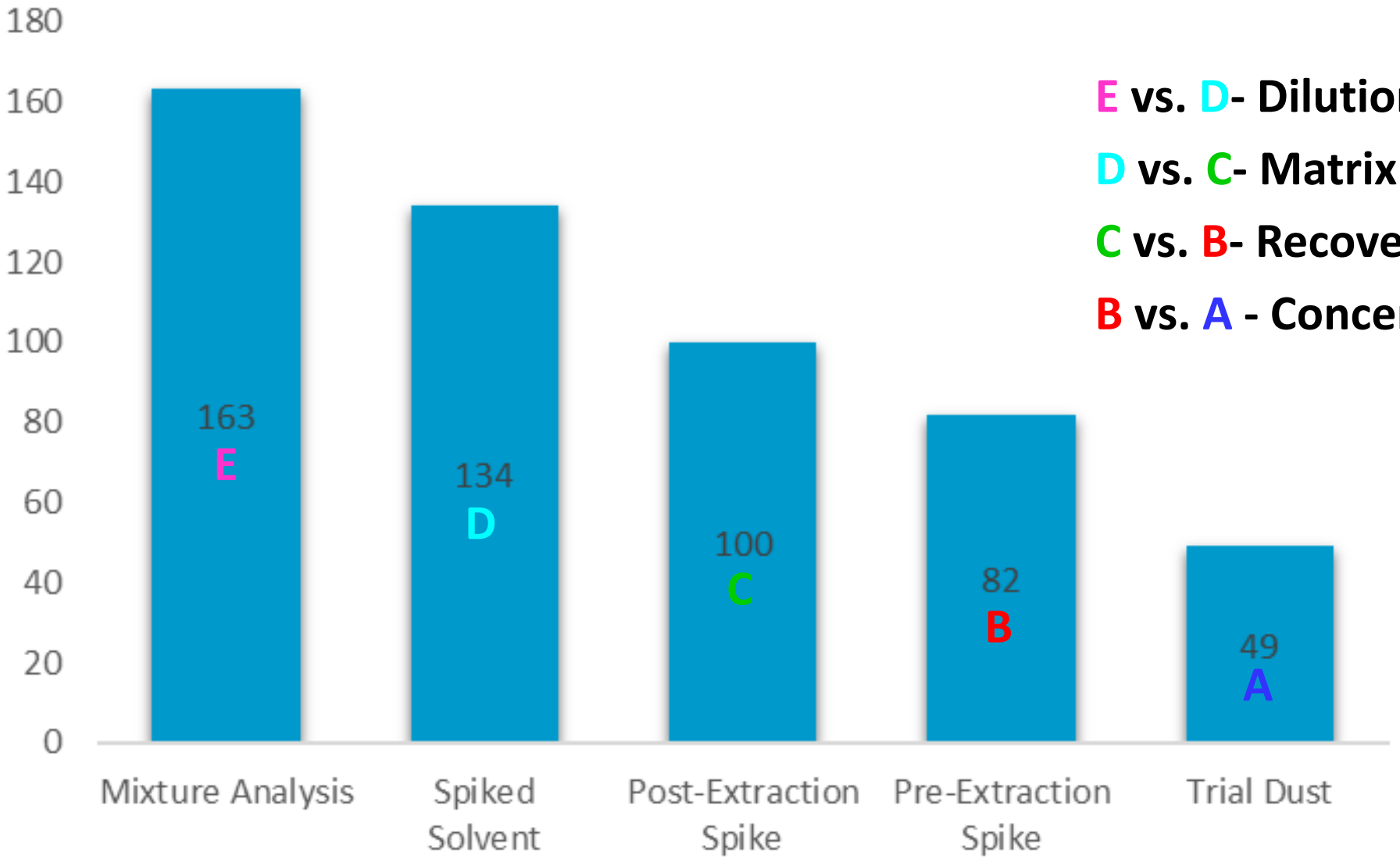
A. ENTACT and **B.** 4× dust spiked before extraction

E. ENTACT mixture

D. Solvent spike

A vs. B- Concentration issues
B vs. C- Recovery
C vs. D- Matrix suppression
D vs. E- Dilution issues

Losses at Each Stage
Mixture Analysis vs. Spiked Solvent



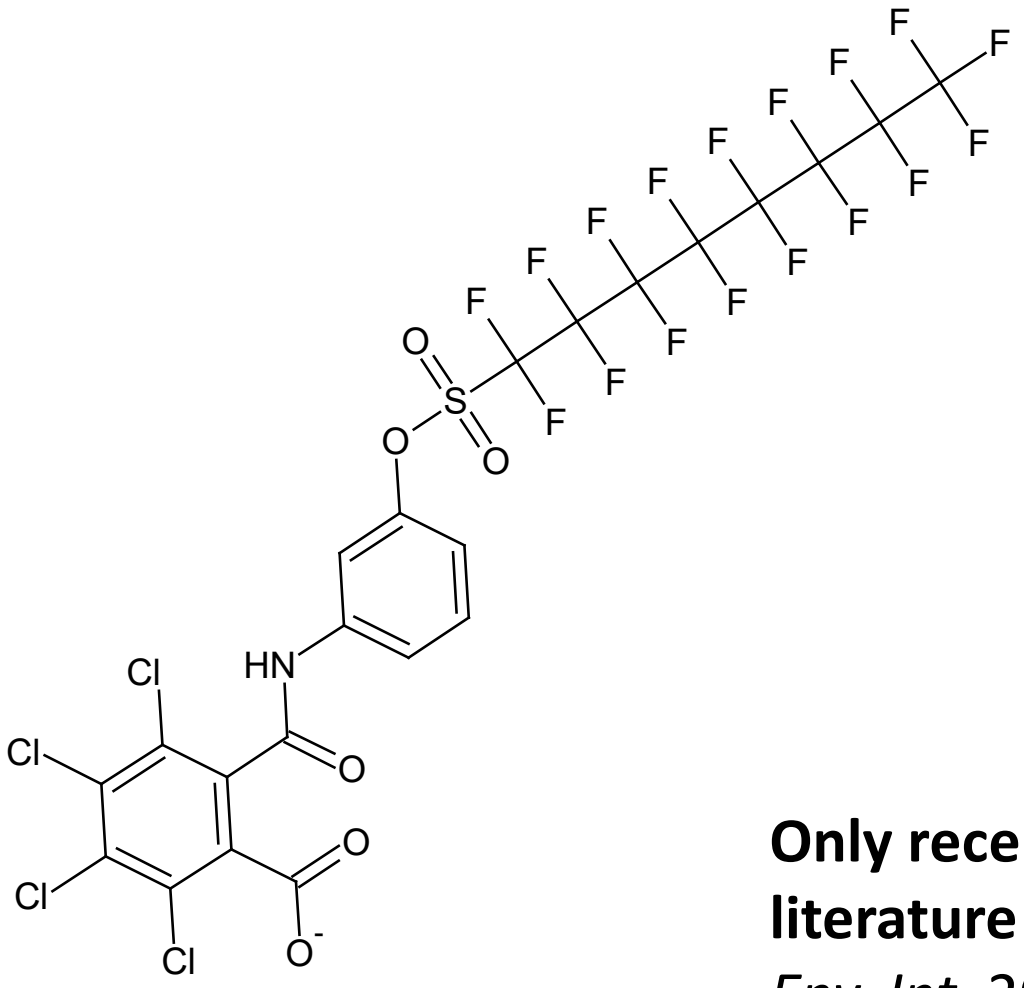
E vs. D- Dilution issues (29)

D vs. C- Matrix suppression (34)

C vs. B- Recovery (18)

B vs. A - Concentration issues (33)

Unknown Compound Identified



2002 - Perfluoroalkyl Sulfonates; Significant New Use Rule (EPA)
2004 - UK Draft List of Compounds Potentially Degrading to PFOS in the Environment

Only recently reported in literature – Zhang et al, *Env. Int.* 2019

35% American House Dust

Rager et al. *Env. Int.* 2016

Post-doc Opportunities at the EPA

- ORISE
  - Oak Ridge Institute for Science and Education
  - https://orise.orau.gov/internships-fellowships/postdocs.html
- ORAU
  - Students or recent grads = “student services contractor”
  - https://www.zintellect.com/Catalog
- Federal Postdoc positions (not offered often)
- NRC

What it’s like to work at EPA

- Pros
  - Great researchers
  - Working towards a cleaner environment
  - More freedom in research than industry
  - Stability and opportunities to advance (for permanent employees)
- Cons
  - Administrative burden (not always on postdocs)
  - Ways of doing things don’t always make sense
  - ORISE funding is renewed yearly

Contributing Researchers

This work was supported, in part, by ORD's Pathfinder Innovation Program (PIP) and an ORD EMVL award

EPA ORD

Hussein Al-Ghoul*
Alex Chao*
Louis Groff*
Jarod Grossman*
Kristin Isaacs
Sarah Laughlin*
Hannah Liberatore
Charles Lowe
James McCord
Jeff Minucci
Katherine Phillips
Tom Purucker
Randolph Singh*
Jon Sobus
Mark Strynar
Elin Ulrich

* = ORISE/ORAU

EPA ORD (cont.)

Chris Grulke
Kamel Mansouri*
Andrew McEachran*
Ann Richard
John Wambaugh
Antony Williams

Agilent

Jarod Grossman
Andrew McEachran

Waters

Aurelie Marcotte